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牛带绦虫早期虫卵的卵膜结构的扫描电镜观察

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摘　　要

通过对牛带绦虫早期虫卵的冷冻断割，做扫描电镜观察，见到带科绦虫虫卵在早期阶段保留了典型的各层膜的结构。虫卵共有七个膜层，实际各层之间是互相沟通与渗透的完整体系。待到虫卵成熟时，绦虫的卵壳才破裂，卵黄细胞及卵黄膜层也有退行性变化，而胚膜（embryophore）及幼虫（六钩蚴 hexacanth embryo）的发育则趋于更完整。

对带绦虫的成熟虫卵的超微结构，国内、外已有过较细的描述（Inatomi, S. 1962; Ishii, Y. 1972; 王松山等 1980）。这些报告多侧重于对带绦虫的胚膜超微结构的研究，尤其是对胚膜微管系统的观察，这些观察对于了解虫卵的生理是很有意义的。此外，Ogren, R. E. (1953, 55, 56, 57, 67) Smyth, J. D. (1956) 曾分别对 Linstowiidae 科绦虫的 *Oochoristria symmetrica* (=O. ratti) 及假叶目绦虫（Pseudophyllidae）的 Schistocephalus solides 的虫卵的发育做了一系列的观察；森山（1961）也对未成熟的牛带绦虫卵做过观察。但这些研究的方法，主要是光学显微镜的观察，对一些虫卵的微细结构，仅是以示意模式图来表示，而不能直接地表达其观察结果。

本文试图应用冷冻断割法（freeze fracture）将牛带绦虫卵剖开，通过SEM观察绦虫卵的早期三维结构，对绦虫卵的膜结构做了进一步地了解。

材料及方法

选用牛带绦虫（Taeniarhynchus saginata）的成熟体节及早期孕节，虫体系用中草药“灭绦灵”（含30%的鹤草酚 Agrimonolide）驱下者。样品材料有两种来源，一种为新鲜驱下的虫体，经清洗后，固定于4%的戊二醛溶液中，立即带回实验室以制备样

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分；另一种为固定保存的标本，保存于醋酸甲醛乙醇固定液中（AFA固定液），保存时间为1－3月，然后带回实验室。以上两种材料均经双蒸馏水清洗五次后，按照田中氏及德永氏（1980）冷冻断割剖出法步骤制备样品。其具体步骤如下：

①1％锇酸固定1小时。②用pH7.4磷酸缓冲液浸洗三次，每次20分钟。③浸入25％的二甲亚砜 dimethyl sulfoxide (DMSO) 中二次，各30分钟。④转浸于50％的DMSO中二次，各30分钟。⑤在液氮冷冻罐中进行冷冻断割。⑥断割后的样品放入50％的DMSO中。⑦双蒸馏水洗五次，每次20分钟。⑧0.1％锇酸中三次，每次10分钟。⑥双蒸水洗三次，每次20分钟。⑩2％单宁酸二次，每次30分钟，⑪双蒸水洗六次，每次10品钟。⑫1％锇酸30分钟。⑬双蒸水洗二次，每次30分钟。⑭丙酮梯度脱水，每级30分钟。⑮醋酸异戊酯置换，2小时或过夜。⑯Co_2临界点干燥。⑰真空喷镀金膜（部分样品用Eikoi BⅡ型离子溅射器镀膜）。⑱用Hs 550型扫描电镜、15Kv，或Hs 450型扫描电镜、25Kv，观察并拍照。以上步骤应严格掌握，若在时间上及步骤上有所变动，常会影响样品的质量。

以上共制备样品120份，经选用观察者20份；共拍制扫描电镜照片108张，选用11张。

此外，将成熟节片及早期孕节片做10μ厚度的石蜡包埋组织的纵切、横切的连续切片，以备对比观察。

观 察 结 果

子宫及子宫腔：孕节纵切的光学检查呈典型牛带孕节子宫侧枝，即16枝以上。宫腔分枝盲端充填虫卵，虫卵具有外壳。扫描电镜低倍放大。可见子宫腔内壁有绒毛层、绒毛下层，最外为肌纤维层（图1）。子宫壁的厚度为10μ，宫腔内虫卵呈杏核状，充填于宫腔内（图2）。虫卵的表面被覆有一层微具褶皱的分泌性物质（图3）。作者认为这种分泌性的物质不是虫卵本身的膜，而是来自于子宫的分泌物，因为在宫腔内可以找到几个虫卵同时被一块膜性的分泌物所覆盖。

虫卵的断割：经剖割的虫卵呈未成熟期的各个阶段（图4、5），表现为具有韧性外壳，胚膜尚未角质化，六钩幼胚处在发育阶段。断割面所示的虫卵可以分为以下各层：卵壳、卵黄膜、卵黄层、胚膜的外层、中层、内层及幼胚的外膜层。共为七层。在卵壳与胚膜层之间有1个或2个卵黄细胞，由卵黄膜将它同胚膜包裹在一起，这种细胞有人称为壳膜细胞（森山，1961），与分泌形成胚膜的物质有关。

卵壳：为单层、均匀的膜状结构，厚度为0.116—0.222μ，这与Inatomi氏测量透射电镜下的成熟虫卵卵壳的厚度0.063μ相差较大。从剖面来看，在卵壳的一端形成一穹窿状的空隙（平面透视则呈半月型），这一空隙即光学显微镜观察下的透明层，实际上，它并不是一个层次，而是一个空腔，空隙中间最长可达6.11μ。空隙内无液体，但有时可见有一些胶性凝物，很可能是在虫卵断割后，处理样品的过程中，使卵内液体逸出。虫卵的另一端，有时可见有突起，突起部的卵壳较厚（图3、6）。

卵黄膜及卵黄层（5、7）：卵黄膜很薄，0.111μ，是一层具网眼状的被膜。膜下

为一层厚薄不一的疏松球状颗粒层，为卵黄层，其厚度为0.3—0.92—1.29μ之间。卵黄细胞与胚膜一起被包裹在卵黄层内，胚膜细胞6.296×8.158μ，断面呈泡球状(图11)。

胚膜，分三层，早期胚膜仅外层及内层为膜质结构，中层为一层球状颗粒。近成熟期的胚膜，总厚度为1.66—3.40μ，外层胚膜为具有筛孔状的膜层，厚度为0.185μ。中层胚膜疏松，内部有微管及凹陷，凹陷的剖出面具有内膜，故应当称做凹陷泡(concavely follicle)(图10)，此即Inatomi氏所指出的凹陷泡及微管系。内层胚膜0.4μ厚，为胚膜的衬里层，该层呈筛孔状。这种结构只有通过虫卵的冷断剖出面的扫描，才能从胚膜的内面看清楚这种结构(图6)。为此，可以确证胚膜与胚膜内腔是沟通的。我们在猪带绦虫早期及成熟虫卵的胚膜内层，也同样地观察到这种筛孔状的结构。

幼虫膜：为一层均匀的透明膜，厚度为0.259—0.77μ，膜表面分化成为一层扁平细胞，胞核紧贴膜壁。膜的外面常见有许多丝状结构与胚膜内层相连，将幼虫固定悬挂在胚膜的内腔中(图8)。

讨　论

Ogren, R. E. (1957) 曾将 Oocheristica symmetrica 虫卵里幼胚的发育分为四个期，他认为可以概括所有绦虫幼胚的发育。即分为①桑椹期，②小钩形成，实质及肌肉发育期，③膜形成及上皮腺 (epidermal gland) 发育，④体细胞形成期，即感染期。由于该氏当时受研究方法的限制，这种人为地划分阶段，并没有反映出幼胚发育的实质。事实上，从牛带绦虫链体脱落端孕节片的子宫分枝中，将其中卵块经过断割后，可以见到各种不同发育阶段的虫卵(图4)。这是牛带绦虫虫卵发育上很值得注意的现象。由于本文没有涉及幼胚的发育问题，但可以指出，采用冷冻断割剖出法来研究幼胚的发育，将会是一种很有效的研究方法。

Smyth, J. D. (1969) 根据圆叶目绦虫 (Cyclophyllidea) 虫卵卵壳的特征将它分为三个类型，即①复孔属类型 (Diphylidium type)，具有薄的卵壳及带丝的蚴虫。犬复孔绦虫、莫尼茨绦虫及膜壳绦虫属于这一型；② Stilesia 型：此型虫卵没有卵黄泡 (vitellaria) 而有被覆层 (cellular covering)；③带绦虫型：虫卵没有卵壳，而具有厚的胚膜。当然，这种分型的办法仅是对成熟的虫卵而言。以带绦虫来讲，它的未成熟虫卵体现了虫卵的原始性的模式结构。它是有卵壳的，而且壳的一端有空腔，另一端常较突出，但绝无像过去所认识的那种在卵壳两端尖棘状突出物。对于有折光性的角质性的胚膜，在它的早期阶段也是膜状结构，在不断地发育过程中方逐渐地分化出来。作者在观察带科其他绦虫(猪带绦虫、细粒棘球绦虫等)的早期虫卵时，也观察到各种虫卵之间，在早期阶段，存在着类似的模式结构特征。

王松山等 (1980) 曾指出，成熟虫卵的胚膜与卵壁之间有很大的间隙，在胚膜的表面附着有球状的致密物质与空泡。笔者认为，这可能是来自卵黄层退行性变化的结果。卵黄层在早期虫卵阶段是一层疏松的球状层，其外被覆一层网状的卵黄膜，这种结构便于营养物质的转送和气体的交换。此外，成熟的带绦虫卵的中胚膜层是由外宽内窄的楔

形柱状体砌成的球形结构，外胚膜层形成所谓小凹陷的孔区。而早期的胚膜并没有形成角质，也没有间隔成放射状的纹理，而是在外胚膜层下方布满一层圆形颗粒物质，这些圆珠状的物质在逐渐发育成熟时，由于互相挤压而形成棱形的胚膜。在早期已出现凹陷和微管，位置在胚膜的中层，还没有移向外方。在胚膜端部的卵黄囊内有一个或两个带有核的泡球状的卵黄细胞，与胚膜一起被包裹在卵黄层内，随着胚膜的发育而逐渐消失，显然它是与分泌形成胚膜的物质有关。

值得注意的是胚膜的内层通过断割后，可以清楚地看到内层里面有许多筛孔通向内腔。由此而知，虫卵各层膜之间是有机的互相沟通、渗透的完整体系。

小 结

冷冻断割牛带绦虫未成熟的虫卵，可以分为壳层、卵黄膜、卵黄层、胚膜的外、中、内三层及幼胚的膜层。这与带科绦虫模式虫卵结构相似。

牛带绦虫卵壳呈杏核状，两端并无棘状突出，一端呈半月型空隙，卵壳外的膜样被覆物系子宫的内壁分泌物形成。

早期的胚膜也分三层，凹陷泡及微管处在中层位置，胚膜内层及外层均呈筛孔状，以沟通内外。

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森山贞治 1961 四吸具类条虫の卵子の构造，并びに胚発育に関する研究（1）缩小条虫卵の構造，并びに胚発育に就いて．寄生虫誌，10：272—278

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STUDIES ON THE ULTRASTRUCTURE OF THE PREMATURE OVA OF *TAENIARHYNCHUS SAGINATUS* GOEZE, 1782. BY SCANNING ELECTRON MICROSCOPE

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In this article, we present the observations of the ova of Taenidae which retain, as seen under scanning electron microscope, the premature ova of the Taeniarhynchus saginatus. An ovum comprises seven layers, which actually interpenetrate and interplay as a complete system. When the ovum becomes mature the capsule breaks, the yolk cells and the vitelline membrane undergo degenerative changes, but the embryophore and the hexocanth embryo grow into completion.

Many detail descriptions have been made at home and abroad of the ultrastructure of the mature ova of Taeniidae (Inatomi, S. 1962; Ishii, Y. 1972; Wang Song-Shan and others 1980). These reports lay particular emphasis on the research of the embryophore's ultrastructure, as the embryophore's tubular system has special importance to our understanding of the physiology of the ova. Our researches, we study the ova of Taeniidae at an earlier stage, investigate its basic structure and ultrastructural changes in its development. It may help us further understand this new problem.

MATERIAL AND PROCEDURE

Choose mature and earlier-stage gravid proglottids, the worm being eliminated by the Chinese herbal medicine "Nie-Tao Ling" (30% Agrimonolide) and by Mebendazole. Keep the newly eliminated worm, when cleansed, in 4 % glutaraldehyde solution for an hour. Now cleanse it once more with double distilled water, immerse it into 25% and 50% dimethyl sulfoxide solution (DMSO). Then prepare samples according to the Tanaka-Tokunaka freeze fracture method. After Co_2 critical-point drying, evaporate gold with a vacuum coater, but to a part of the samples apply Eikoi BⅢ ion sputling coating. Finally, observe wilh Hs-450 and Hs-550 scanning electron microscopes, 25 Kv and 15 Kv respectively.

Observe 20 freeze fractural samples of mature and gravid proglottids in total. Furthermore, make the mature and gravid proglottids into paraffin sam-

ples and make a series of longitudinal and transverse sections (10μ in thickness).

RESULTS

UTERUS AND UTERINE CAVITY: The optical observations of the gravid proglottids show classic uterine lateral branches of the Taeniarhynchus saginatus, i. e, more than 16 lateral branches, and the blind end of the uterine cavity is filled with ova which have capsules. The low-power magnification by the scanning electron microscope shows that the inner wall of the uterine cavity consists of chorionic membrane, subchrionic layer and the outmost layer of muscular fibriles. The ova packed in the uterine cavity are in the shape of an apricot kernel, and nodulous protrusion is often seen on one end of the ovum whose surface is covered with slightly folded membraneous substance.

FRACTURE OF THE OVA: The chopped ova shows immaturity by its transparent elastic outer shell, its unkeratolized embryophore and its oncosphere being at an early stage of development. The ova comprise 7 layers: the capsule, the vitelline membrane, the vitelline layer, the outer layer of the embryophore, the median layer and the inner layer, the inner lining of the oncosphere.

The capsule: Single-layer membrane, homogenous structure with a thickness of 0.116–0.222μ, which has a great difference from the 0.063 μ measurement of a mature egg's capsule taken under an electronic microscope by Inatomi. One end of the ova forms a dome-like gap with a crescentshaped plane, and without any liquid in it similar to an air sac. On the other end nodulous protrusion is often seen.

The vitelline membrane and vitelline layer: The vitelline membrane is very thin (0.111 μ). It is a mesh-like clothing membrane, beneath which there is a loose layer of ball-like material, i. e. the vitelline layer, its thickness being 0.3–0.92–1.29 μ.

The vitelline cell: It is in the vitelline sac, 6.295×8.518 μ. Its section is in the shape of a bulb.

The embryophore: It comprises 3 layers which have a total thickness of 1.66–3.24 μ. The outer layer is a meshed membrane with a thickness of 0.11–0.185 μ. The median layer is of loose texture with round concavity follicles inside, and also irregular gaps which are referred to by Inatomi as small concavities and tubular system. This structure can only be seen from inside the embryophore by scanning the fracture. The observations prove that the tubular system of the embryophore is linked up to the inner cavity.

The oncosphere membrane: It's a homogenous membrane with a thickness of 0.259–0.77 μ. There are many tube-like objects which link to the inner wall

of the embryophore, thus the oncosphere is suspended in the inner cavity.

DISCUSSION

The premature ova of Taeniarhynchus saginatus retains the original typical structure of an ovum. The ovum has a capsule, a cavity in one end and a nodulous protrusion on the other. In comparison with the refractive keratotic embryophore of a mature ovum, it is not yet keratolized at the early phase, and it has neither any intermission nor radiative strix which are formed in the course of later development. Wang Song-Shan et al. pointed out that there is an intermission of a considerable size between the embryophore and the capsule of a mature ovum, and on the surface of the embryophore are the ball-shaped dense bodies and vesicles. These might be the result from changes of the vitelline layer. This layer consists, at an earlier stage, of loose material of ball-shaped bodies and outside of it is the vitelline membrane. This structure is convenient for the transfer of nutrition and the exchange of air. The embryophore in the median layer of a mature ovum is a ball-shaped structure built with wedge-shaped columns with their bigger ends out. The outer layer bears mashed areas of concavities which are in the median layer of the embryophore at the earlier stage, not yet moved outward then. Special attention should be paid to the fact that, in the inner layer of the embryophore, there are many meshes which link up the inner cavity. In view of this, we can say that the whole ova, with all its layer *interpenetrating* and interplaying, is a complete system.